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Preliminary

Purification of Xyloglucanase from Auxin-treated Pea Stems*[1]

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Endo-1, 4-β-glucanase activities responsible for degradation of xyloglucan are associated with the auxin-induced expansion of cells in pea stems[1]. The enzymes hydrolyze the internal 1, 4-β-glucosyl linkages of derivatives of cellulose, *e.g.* carboxymethylcellulose, cellodextrin and xyloglucans. Recently, two endo-1, 4-β-glucanases have been isolated in suspension-cultured popular cells, which specifically cleaved the 1, 4-β-glucosyl linkages of carboxymethylcellulose, swollen cellulose and lichenan but hardly degraded xyloglucan. The molecular sizes of these enzymes were 50 kDa[2,3]. Another type of endo-1, 4-β-glucanase (46 kDa) has been obtained from the cell wall preparations of auxin-treated pea stems, of which molecular size is smaller than that (70 kDa) reported in earlier studies[4]. There must be several forms or isozymes of endo-1, 4-β-glucanase in pea stems after treatment with auxin. This paper describes the charactrization of a xyloglucan-specific endo-1, 4-β-glucanase in auxin-treated pea stems.

A xyloglucan-specific endo-1, 4-β-glucanase was purified to apparent homogeneity from the auxin-treated segment of pea stems by sequential cation-exchange chromatography. The purified enzyme gave a single protein band on SDS-PAGE. The isoelectric point (pI) was over 8. The apparent molecular size was 77 kDa on SDS-PAGE, whereas it was 70 kDa on gel filtration. The enzyme may be glycoprotein located in the cell wall and extracellar space of the stems because the activity was recoverd from the extracts of the wall preparation plus the apoplastic solution. The enzyme specifically cleaved the 1, 4-β-glucosyl linkages of xyloglucan backbone, but it did not hydrolyze carboxymethylcellulose and had no transglycosylation activity for xyloglucan. It is concluded that the xyloglucanase activity has been separated from an endo-1, 4-β-glucanase (cellulase) in auxin-treated pea stems.

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*[2] Labolatory of Gene Expression.

The enzymatic reaction has been defined as a specific xyloglucanase, which hydrolyzes internal linkages adjacent to unsubstituted glucose units of both unfucosylated and fucosylated, thereby introducing free reducing end-groups at these positions. It has neither a hydrolase activity on carboxymethylcellulose nor transglycosylase reaction on xyloglucans. Therefore, the enzyme is different from either a cellulase from auxin-treated pea stems[4] or an endo-1, 4-β-glucanase from germinating nasturtium seeds[5].

The enzyme seems to be a new wall-loosening enzyme in the cell walls of higher plants and to be present at a very small amount in pea stems, although the enzymatic activity is potentially greatly enhanced by auxin. An enzyme preparation from the cell walls of plant cells always degrades xyloglucan by a two-step mechanism[6], in which xyloglucan is hydrolyzed into oligosaccharides at the first step. The formation of xyloglucan oligosaccharides by xyloglucanase should enhance the degradation and solubilization of xyloglucan in the wall by the action of xyloglucan endotransglycosylase[7].

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